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Absolute Configuration, Optical Activity and Raman Microscopy of L and D-Glutamic Acid

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Amino acids are the building blocks of proteins. Among this diverse group of molecules, glutamic acid (Glu - $C_5H_9NO_4$) plays a key role as a component of enzymes, either with a structural role or as part of the catalysis in the active site to perform biological function [1]. Charged transfer RNAs are loaded with L stereoisomers, so proteins synthesized by the ribosome are exclusively comprised of L-amino acid residues. However, D-Glu is used in formation and development of bacterial peptidoglycan [2]. The determination of the absolute configuration of chiral compounds is critical in many fields of science and technology. In this work, we carried out single-crystal X-ray diffraction (SCXRD) experiments with Cu K_α radiation to determine the absolute configuration of glutamic acid in both enantiomeric forms (L/D). L and D-Glu isomers were also analyzed to determine their optical activity and structural information.

The amino acids were purchased from Sigma-Aldrich and used without further recrystallization. The crystallization experiments were: 1) 50 mmol of L-glutamic acid (≥ 99% pure); m.p.: 206-208 °C. ^{1}H NMR (D_2O, DSS, 400 MHz): δ = 2.14 (m, J = 7.2 Hz, 2H, H_a), δ = 2.55 (m, J = 8,8 Hz, 2H, H_b), δ = 3.80 (t, J = 6.8 Hz, 1H, H_c) was dissolved in 1 mL of ultrapure water (Milli-Q water, Merck Millipore); 2) 50 mmol of D-glutamic acid (≥ 99% pure); m.p.: 210-211°C. ^{1}H RMN NMR (D_2O, DSS, 400 MHz): δ = 2.14 (m, J =7.2 Hz, 2H, H_a), δ = 2.55 (m, J = 8 Hz, 2H, H_b), δ = 3.80 (t, J =6,4 Hz, 1H, H_c) was dissolved in 1 mL of ultrapure water. SCXRD experiments of L-Glu and D-Glu were collected on a Bruker D8-QUEST diffractometer with a Cu K_α microfocus source (λ=1.5418 Å) equipped with a CMOS detector (300 (2) K). Optical activity of L-Glu and D-Glu was done on a Jasco 710 spectropolarimetrer (Jasco Inc., Easton MD, USA) on a 0.2 cm^3 quartz with 1 mm path-length containing 20 mM Tris-HCl pH 8.0, 20 mM NaF and 6 mM of each L-Glu/D-Glu. A separate experiment was done with a mixture of 3 mM DL-Glu each. Data was reported as positive or negative ellipticity *vs.* wavelength. Solid-state Raman spectra were taken in a Horiba Jobin-Yvon LabRam HR high-resolution Raman microscope, equipped with a charge-coupled device detector and an excitation laser source with a wavelength of 632.8 nm.

After refinement of the Flack parameter [3], the absolute configuration was determined. L-Glu had an absolute configuration "sinister - S" with a Flack parameter value 0.03 (3), whereas D-Glu had an absolute configuration as "rectum - R" and Flack parameter of 0.09 (4) (Figure 1). The optical activity of each enantiomer was evaluated using circular dichroism. An opposite optical activity was found for the two enantiomers (Figure 2a). As expected, an equimolar mixture of L-Glu and D-Glu gave a near zero ellipticity for the 200-260 nm range. Therefore, L-Glu is classified as levorotatory (-) and D-Glu as dextrorotatory (+). Raman spectra showed the main structural characteristics of the compounds, from

3021 to 2931 cm^{-1} the stretching modes of the -NH_3 and -CH_n groups are observed, meanwhile the existence of the zwitterionic form of the glutamic acid is confirmed by the bands at 1658 and 1406 cm^{-1} of the carboxylate group.

In summary, the absolute configuration and the optical activity of the entitled compounds have been established.

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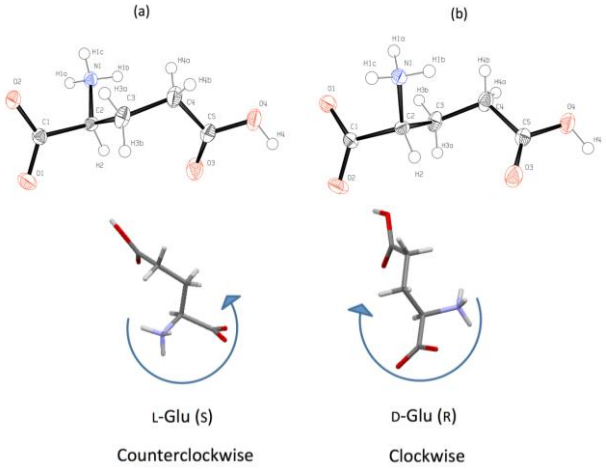

Figure 1. Ortep representation of the asyymetric units of: a) L-Glu and b) D-Glu along with their respective absolute configuration. (atoms are drawn as 50% probability ellipsoids).

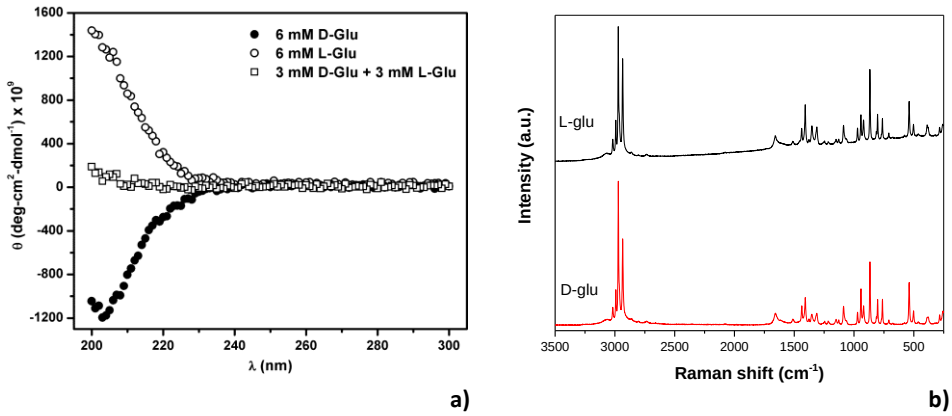

Figure 2. a) Circular Dichroism Spectra of L and D-glutamic acids and the racemic equimolar mixture and b) Raman spectra of L and D-glutamic acids.

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